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Docket No.: NEL-0006 (80283-0014) PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Jonathan P. WONG et al.

Application No.: 10/091,567

Filed: March 7, 2002

For: DNA VACCINE USING LIPOSOME-ENCAPSULATED PLASMID DNA ENCODING FOR HEMAGGLUTININ PROTEIN OF INFLUENZA VIRUS

Confirmation No.: 7851

Art Unit: 1648

Examiner: Myron G. Hill

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Jonathan P. Wong, being duly warned, hereby declare and say:

1. I received a B. Sc in Biochemistry from the University of Saskatchewan, Saskatoon, Saskatchewan. I received an M.S. in Biochemistry from the University of Saskatchewan.

2. I am presently employed as a Defence Scientist for Defence R&D Canada – Suffield and have been employed since July 20th, 1987 by the Minister of National Defence of Canada.

3. I am one of the inventors of the present invention and am familiar with the technology involving the present patent application, and have thoroughly reviewed the application

as well as the prior art cited by the U.S. Patent and Trademark Office, particularly articles Immunobiology, p. 21 - 30 (1999) by Sha et al. ("Sha") and Promega Technical Bulletin 206, rev. 7/1999 ("Promega").

4. From my analysis, the object of the claimed invention is to demonstrate the novelty and efficacy of a DNA vaccine against influenza comprising of a liposome-encapsulated plasmid DNA containing a gene encoding the hemagglutinin protein of the influenza virus. This novel DNA vaccine is encapsulated in liposomes and has been shown to elicit antiviral immune response that completely protect mice against a lethal challenge of influenza virus.

5. Sha et al. cited by the Examiner teaches the use of a plasmid/liposome mixture to induce mucosal immunity. In that approach, the plasmid is mixed with a commercially available liposome made from Dosper (materials and methods, p. 22, line 8-10). The plasmid formed a *complex* with the liposomes. This is a DNA/liposome mixture. The present invention relates to the use of a liposome-encapsulated plasmid DNA where the plasmid DNA is encapsulated within liposomes. The liposome formulation, method, and the resulting structure of the plasmid in liposomes are fundamentally different. The present invention describes the use of plasmid DNA which is encapsulated in liposomes, rather than a plasmid DNA *complex*.

In Sha et al., the approach was used to induce mucosal immunity in the respiratory tract, and was shown to be ineffective in protecting mice against influenza virus challenge (results, p. 26, line 1-6, last paragraph). On the other hand, the present invention uses liposome-encapsulated plasmid DNA to protect mice against lethal challenge with influenza virus infection. The present patent application provides unambiguous scientific evidence that liposome-encapsulated DNA vaccine provided 100% protection to mice against influenza virus challenge (p 14, lines 5-14 of Disclosure, and Figures 3 and 4). These completely different results show that the two approaches are entirely different, and suggest that encapsulation of plasmid DNA within liposomes (rather than complexing with liposomes) is critically important in offering influenza virus protection.

Thus, it is clear that the present invention is vastly different and more superior to the plasmid/liposome complex described by the Sha et al. reference as this present invention discloses a liposome-encapsulated DNA vaccine that offers 100% protection against influenza virus infection,

in contrast to a liposome/plasmid DNA complex that does not offer any level of protection against influenza virus infection.

6. The Promega catalogue teaches that pCI is a mammalian expression vector which is designed to promote the expression of DNA inserts in mammalian cells. The transfection of the DNA in mammalian cells may be mediated by many reagents, including cationic lipids. The Promega catalogue, by itself or together with Sha reference, does not teach that pCI vector is capable of being used as a vaccine candidate or as an expression system for the influenza heamagglutinin protein as a DNA vaccine candidate.

As the present invention describes use of the liposome-encapsulated pCI plasmid with an inserted hemagglutinin gene as a DNA vaccine candidate to elicit a potent antiviral immune responses to influenza virus, it is clear that this present invention is different and distinct from the teaching of the Promega Catalogue.

7. As shown in the two diagrams below (same referenced Figures 3 & 4 as disclosed in the patent specification), there are clear and unambiguous evidence that deoxyribonucleic acid (DNA) vaccine comprising a liposome-encapsulated plasmid containing a gene encoding for hemagglutinin protein are superior to the ones disclosed in the Sha et al. reference.

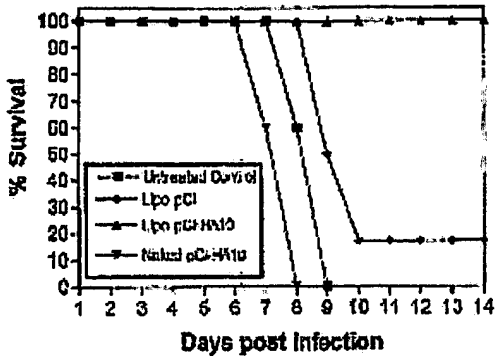

Fig. 3 The efficacy of intranasally administered liposome-encapsulated pCI-HA10 against influenza virus in mice. Mice intranasally immunized with one primary and three booster doses of liposome-encapsulated pCI-HA10 (Lipo pCI-HA10), naked pCI-HA10 or liposome-encapsulated pCI. At 1 week post final immunization boost, the mice were intranasally challenged with 5 LD_{50} of virus. The survival rates were monitored daily.

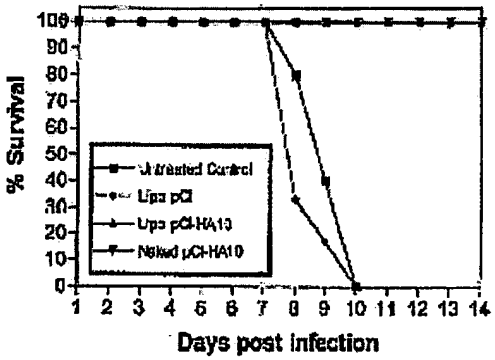

Fig. 4. The efficacy of intramuscularly injected naked and liposome-encapsulated pCI-HA10 to protect mice against respiratory lethal influenza virus challenge.

Quoting from page 14, lines 4 to 16 of the Disclosure (under the sub-heading of "DNA Vaccination"), the efficacy of naked and liposome-encapsulated pCI-HA10 to protect animals against lethal challenge of influenza virus by intranasal and intramuscular administrations is shown in Figs. 4 and 5. Non-immunized mice succumbed to the influenza infection at early as 7 days post infection, and all animals were dead by day 9. All mice which received intranasal immunization with naked unencapsulated pCI-HA10 also succumbed to the infection, with no increase in survival rate nor survival time (Figure 3). In contrast, mice immunized intranasally with liposome-

encapsulated pCI-HA10 were found to be completely protected with 100% survival rate ($p < 0.01$ vs. control or naked pCI-HA10 group).

When the pCI-HA10 DNA was administered by intramuscular injection, both liposome-encapsulated and naked pCI-HA10 plasmid were shown to provide complete protection against the virus challenge (Figure 4). In contrast, liposome-encapsulated pCI without the HA insert provided little or no protection.

As stated earlier, results from these efficacy studies are in complete contrast to the efficacy study shown by the Sha reference using plasmid DNA/liposome complex. By using a vastly different approach, the Sha study showed no protection against influenza infection. These results confirm scientifically the distinctness of this present invention from the Sha reference, and therefore warrant serious re-consideration by the Examiner.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Dec 17th, 2003
Date

Jonathan P. Wong